Remarks

Claims 1-8 are under consideration. Claims 1, 2, 3 and 6 have been amended. Claims 9-11 have been cancelled. Reconsideration of claims 1-8 is respectfully requested.

35 U.S.C. § 112, first paragraph

The Action rejects claims 1 and 2 under 35 U.S.C. 112, first paragraph, as failing to comply with the written description requirement specifically in relation to the terms "c-23" in claim 2 and "B6"in claims 1, 2, 4, 5, 7, and 8.

In response, applicants have amended claim 2 to correct the error from c-23 to c-2J.

In reference to "B6" applicants introduced this term in the January 18, 2005 by deleting "C57". This is because one skilled in the art uses "C57", "B6" ,"C57BL/6J" and C57BL/6J-Tyr $^{c\text{-}2J}$ interchangeably. No new matter is added.

The Action states that *"The specification does not teach how to introduce B6 cells into C57 blastocysts"*

In response, applicants refer the Examiner to page 13, line to page 15, line 6 which describes in detail the introduction of ES cells into blastocyts. The Examiner is requested to review the Specification more carefully.

35 U.S.C. § 112, second paragraph

Claims 1,2,3 and 6 were rejected under 35 U.S.C. 112, second paragraph as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicants regard as the invention because claim 1 has a typographical "form" instead of "from".

In response, claim 1 is amended to correct the informality.

Claim 2 is amended to clairify B6 ES cell line for C57BL/6J-Tyr $^{c\text{-}2J.}$

Claim 3 is amended to clarify " said Chimeric mouse and to correct "been" to "be".

Claim 6 is amended to correct "coar" to "coat".

Therefore , based upon the above amendments the above rejections should be withdrawn.

Claim Rejections under 35 U.S.C. § 103

The Action rejected claims 3-8 under 35 U.S.C. 103 (a) as being unpatentable over Schuster-Gossler, et al (2001, BioTechniques, 31:1022-1026) and Smith (2001, Annu. Rev Cell Dev Biol 17: 435-462) in view of Katayama et al. (2001, Biochemical and Biophysical Research Communications 281: 1134-1140).

The Action states on Page 9, that *"Applicant's arguments with respect to claims 1-8 have been considered but are moot in view of new grounds of rejection. The Examiner has written a new rejection."*

In response, applicants request an explanation as to why a new rejection was written, especially since the one of the two new references cited in the above rejection

(Smith) was submitted in the Information Disclosure Statement and discussed in the Background Section of the Specification. See Page 3, lines 12-16.

MPEP 707.07(g) specifically requires that "Piecemeal examination should be avoided as much as possible". The Examiner should also specifically clarify in the record that the reference cited in the 10/20/04 Office Action- Wei (1997, Annu.Rev.Pharmacol.Toxicol., 37:119-41), is withdrawn.. By not providing a complete evaluation in the 10/20/04 Office Action, the Examiner has caused applicants to incur extra prosecution expenses and delay the allowance of the patent.

1 (Page 5, line 1 to pg 6 line -10). The 4/20/2005 Action repeats word for word form the Office Action mailed October 20, 2004 that: " *Schuster-Gossler et al teach that while gene-targeted mice means of homologous recombination are a valuable tool, the efficient production of them have not always been met. One reason for this inefficiency results from finding the best host blastocyst/ES cell line combination that yields chimeric animals with germline transmission. To generate a transgenic animal, one would carry out the genetic manipulation in the ES cell, introduce the ES cell into a host blastocyst and allow the embro to develop into a chimeric animal.* ***One way of discriminating whether a cell originated from an ES cell or the blastocyst host is via a genetic marker of the cell, e.g., coat color.*** *(emphasis added) Because some ES/transgenic cells may have become germline cells, one would breed the chimeric animal and select the mice that have been derived from the ES cells. Schuster-Gossler, et al isolated cells from C57BL/6J (B6) mice (page 1022, second column, "B6 ES Cell Derivation and Culture," lines 1-8). B6 ES cells that had gone through 9-17 passages were thawed from liquid*

nitrogen and then injected into blastocysts from a coisogenic mouse, c ^{2J}, or a noncoisogenic mouse, FVB (page 1022, third column, "Generation of Chimeric Mice," lines 1-9). ***Coat color was used to determine whether the cells had come from the ES cell (black) or from the blastocysyt (white)*** *The male mosaic mice were then mated to , c ^{2J} female mice to determine the germline transmission (page 1023, third column, first paragraph, lines 7-11). The ability of the host blastocyst to colonize ES cells was determined. It was found that when the B6 ES cells were injected into the coisogenic blastocyst, more mice had a higher degree of chimerism that had been contributed by the ES cell (i.e., more of their body was black). When the chimeric mice were bred, all but one mouse that were made with B6 ES cell/, c ^{2J} blastocyst (coisogenic), i.e., the same genetic background had had ES –derived offspring (black). However, only 2 of 14 mice made from B6ES/FVB blastocyst (non-coisogenic) produced ES cell-derived mice (page 1025, second column, lines 17-21). Furthermore, coisogenic mice were transmitting the ES lineage more frequently than the non-coisogenic mice (page 1025, second column, line 22 to third column, line 7). While Schuster-Gossler et al teach that other strains of mice can be used to generate transgenic mice and that using host blastocysts that are coisogenic with the ES cell produces more viable ES cell-derived mice. However, Schuster-Gossler et al do not teach how to make transgenic mice.* (emphasis added)

2. (Page 6, lines 11-16) The Action states that "*Smith teaches that C57BL/6 blastocysts are good blastocysts to use in generating chimeric mice because transgenic mice generated from C57BL/6 blastocysts are comprised of high ES cell contribution and*

increased frequency of germ line transmission (Smith, page 438, 2nd par under " Embryonic Stem Cell Derived Mice" lines 12-16).

3. (Page 6, lines 17-23) The Action states that *Kitayama et al teach how to make transgenic mice using C57BL/6ES cells. Kitayama et al teach that C57BL/6ES cells were electroporated with a targeting vector pD2CPRTVS, which was comprisied of a Cre gene and a mutated gene ligand binding domain of the human progesterone receptor gene. For selection of ES cells that underwent homologous recombination, the neo gene was used (Kitayama et al., page 135-136, Materials and Methods, "Targeting vector construction" and "ES cell culture").*

4. (Page 7, lines 1-5) The Action concludes *"... it would have been **prima facie obvious** to one having ordinary skill in the art at the time the invention was made to introduce a transgene into a C57BL/6 ES cell in a method taught by Kitayama et al., and to implant C57BL/6 ES cell comprising the transgene into a C56BL/6 blastocyst, in a method taught by Schuster-Gossler et al.*

Response-Applicants find the above statement is factually incorrect because the method taught by Schuster-Gossler et al does not teach that C57BL/6 ES cell is implanted in C57BL/6 blastocysts . Instead it teaches that C57BL/6 ES cell is implanted in coisogenic C57BL/6J-Tyr $^{c\text{-}2J}$ blastocysts.

Kitayama et al teaches that C57BL/6 ES cells are implanted in ICR mice embryos.

5. (Page 7, lines 6-11) The Action continues *"One having ordinary skill in the art would have been **motivated** to inject* a C57BL/6 ES cell comprising a transgene into a C57BL/6 blastocyst, as taught by Schuster-Gossler, *et al., in order to obtain a transgene C57BL/6 mouse. **Further motivation** is provided by the teachings of Smith that ES cells contribute more to the mosaicism of transgenic mice and thus increase the frequency of germ line transmission.*

Response-Applicants find the above underlined statement is factually incorrect because the method taught by Schuster-Gossler et al does not teach that C57BL/6 ES cell is implanted in C57BL/6 blastocysts . Instead it teaches that C57BL/6 ES cell is implanted in coisogenic C57BL/6J-Tyr $^{c-2J}$ blastocysts.

Regarding Smith, the above statement is misleading because the examiner cited Smith in Paragraph 2 above, as follows: "*Smith teaches that C57BL/6 blastocysts are good blastocysts to use in generating chimeric mice because transgenic mice generated from C57BL/6 blastocysts are comprised of high ES cell contribution and increased frequency of germ line transmission."*

6. (Page 7, lines 12-20)The Action continues *"There would have been **a reasonable expectation of success** given the results of Kitayama et al teaching that a transgene construct could be introduced to C57BL/6 ES cells, in order to generate transgenic mice, and the results of Schuster-Gossler, et al., for teaching that C57BL/6 chimeric mice are more often generated when the ES cell and blastocyst are both from the C57BL strain. A reasonable expectation of success that one will obtain the transgene mouse is also apparent as Smith teaches that mice grown from a C57BL/6 blastocyst are*

more likely to be comprised of the ES cell and that there is increased frequency that germ line transmission occurs when using a C57BL/6 blastocyst".

Response-Applicants find the above underlined statement is factually incorrect the examiner has mistakenly concluded that the C57BL/6 mice (ES cells) and C57BL/6J-Tyr $^{c-2J}$ are the same strain. The method taught by Schuster-Gossler et al does not teach that C57BL/6 ES cell is implanted in C57BL/6 blastocysts . Instead it teaches that C57BL/6 ES cell is implanted in coisogenic C57BL/6J-Tyr $^{c-2J}$ blastocysts.

Comparison of 3 prior art references and present invention

The different elements of the 3 prior art references cited and the present invention are represented in the Table below:

Schuster-Gossler	Smith	Kitayama	Present invention
B6 ES cell lines(black)	-	C57BL/6 ES cells	B6 ES cell lines(black)
Blastocysts c-2J & FVB C57BL/6J-Tyr $^{c-2J}$(white)	C57BL/6	ICR mouse	C57BL /6 blastocysts

Schuster-Gossler used ES (black) cells from C57BL/6J mice, and injected them into blastocysts of C57BL/6J-Tyr $^{c-2J}$(white). As described in the cited section of Schuster-Gossler (in bold) above, One way of discriminating whether a cell originated from an ES cell or the blastocyst host is via a genetic marker of the cell, e.g., coat color. Schuster-Gossler used ES (black) cells from C57BL/6J mice, and injected them into blastocysts of C57BL/6J-Tyr $^{c-2J}$(white). Coat color was used to determine whether the cells had come from the ES cell (black) or from the blastocyst (white).

In fact, one skilled in the art, like Schuster-Gossler, uses coat color as a genetic marker, when introducing ES cells into the cysts of mouse blastocysts by injection, to determine the percentage of the injected ES cells contributing to the host blastocysts. No artisan thinks about injecting the same color ES cells into the same color blastocysts- this teaches away from all of the cited references above because coat color is a very good genetic marker.

However, there are several disadvantages with Schuster-Gossler's method :

First, C57BL/6J-Tyr $^{c\text{-}2J}$ mice are expensive and not available in large quantities for experimentation because only one vendor, the Jackson Laboratory, in Maine, provides this strain in limited quantity. See specification page 4, lines 14 to 21.

Second, maintaining an albino blastocyst donor colony will be needed. Both of them will increase the production costs.

Third, applicants have found that it's very difficult to get gene germline transmission which is the most important step for generating genetically modified C57 mice.

Fourth, even if germline transmission occurs, extra mating has to be needed to remove the tyrosinase mutation. The tyrosinase (Tyr) gene encodes the enzyme tyrosinase that catalyses the conversion of L-tyrosine into DOPA (3,4-dihydroxyphenylalanine)-quinone. The albino mutation abrogates functional activity of tyrosinase resulting in deficiency of melanin pigment production in skin and retina. Tyr maps to a region in the central position of Chromosome 7 that contains a skin tumor-modifier locus. Saran et al. isolated the albino mutation in transgenic mice to assess a possible role of Tyr gene in two-stage skin carcinogenesis. Transgenic expression of the

functional Tyr (Cys) allele in albino mice (Tyr (Ser)) caused a reduction in skin papilloma multiplicity, in four independent experiments and at three dose levels of DMBA (9,10-dimethyl-1, 2-benzanthracene). In vitro mechanistic studies demonstrated that transfection of the Tyr (Cys) allele in a human squamous cell carcinoma cell line (NCI-H520) increases tyrosinase enzyme activity and confers resistance to hydrogen peroxide-induced oxidative DNA damage. These results provide direct evidence that the Tyr gene can act as a skin cancer-modifier gene, whose mechanism of action may involve modulation of oxidative DNA damage. (Saran A., et al. 2004, Oncogene 20:4130-4135). In addition, C57BL/6J-Tyr $^{c-2J}$ mice exhibit delayed development of sweat glands compared with their pigmented littermates (Tian H., et al. 2000, J. Neurosci. 20:7362-7369).

As described in the specification, finding the best combination of host blastocyst / ES cells has been a major obstacle for generating genetically modified C57 mice. See Specification . The present invention has found the solution to finding the best combination of host blastocyst /ES cells i.e., by injecting B6 ES cells into black C57BL/B6 blastocysts. However, coat color cannot be used as a genetic marker and, chimeras need to be identified by genotyping, so do all F1 pups.

Prima facie Obviousness rejection-legal standard

To establish a prima facie case of obviousness three basic criteria must be met. First, there must be some suggestion or motivation, either in the references themselves or in the knowledge generally available to one of ordinary skill in the art, to modify the reference or to combine reference teachings. Second, there must be a reasonable

expectation of success. Third, the prior art references must teach or suggest all the claim limitations. The teaching or suggestion to make the claimed combination and the reasonable expectation of success must be found in the prior art, and not based on applicant's disclosure. In re Vaeck, 947 F.2d 488, 20 USPQ2d 1438 (Fed. Cir. 1991) MPEP 2143.

Response- First, there is no suggestion or motivation in the references or knowledge available to one skilled in the art to implant B6 ES cell into B6 blastocysts. The cited sections in the Action do not support the conclusions the examiner had drawn. Second, there is no expectation of success achieved by the applicants described in the cited references. Third, the cited references do not teach or suggest all the claim limitations.

"There are three possible sources for a motivation to combine references: the nature of the problem to be solved, the teachings of the prior art, and the knowledge of the persons of ordinary skill in the art" In re Rouffet, 149 F 3d 1350, 1357, 47 USPQ 2d 1453, 1457-58 (Fed Cir 1998) (The combination of the references taught every element of the claimed invention, however, without a motivation to combine, a rejection based on a prima facie case of obviousness was held improper). The level of skill in the art cannot be relied upon to provide the suggestion to combine references. Al-Site Corp v VSI Intel Inc., 174 F3d 1308, 50 USPQ 2d 1161 (Fed Cir 1999). " When the motivation to combine the teachings of the references is not immediately apparent, it is the duty of the examiner to explain why the combination of the teachings is proper". Ex parte Skinner, 2 USPQ 1788 (Bd. Pat. App. & Inter. 1986). MPEP 2143.01.

Therefore, as a matter of fact and law, the above rejection should be withdrawn.

In the present invention, applicants have overcome the disadvantages and problems encountered by Schuster-Gossler et al of low blastocyst production in the C2J strain. Applicants discovered the source of the problem causing inefficient germline transmission and production of chimeric mice. Whereas, Schuster-Gossler et al describes a "black into white" chimerism, applicants used a new approach to demonstrate chimerism of chimeras by injecting black B6 ES cells into black B6 blastocysts ("black in black"). Other disadvantages of Schuster-Gossler et al.'s method were discussed above.

Therefore, as a matter of fact and law, the rejection of claims 3-8 should be withdrawn.

7. (Page 8, lines 1-4)The Action also states that *"With regard to the issue concerning coat color as being encompassed by the claims, it is noted that the art does not teach the making of transgenic mice wherein the source of the ES cells and blastocyst are from mice that have the same coat color.*

Response- Applicants interpret the above statement as admitting that applicants invention is novel and unobvious.

The Action then states that (Page 8, lines 4-12) *" However, neither the specification nor the art teaches any biological reason why an artisan would preferentially select coat color for generating transgenic mice. Neither the specification nor the art teach that coat color is one of the reasons that an artisan cannot generate transgenic mice, nor does the art or specification teach that coat color is the basis for gross variation of phenotypes between mice comprising a disruption in a gene of interest. It is understood in the art that using ES cells and blastocysts from different colored mice is a rapid way of identifying the lineage of the generated tissue of the mouse."*

Response- Applicants do not disagree with the above observations. However, applicants have found that because of the above practice of using coat color as the genetic marker, artisans have missed the important discovery that applicants have made, that by using ES cells and blastocysts from the same mouse strain (C57BL/6) they achieved greater success with genetic modification of C57 mice. Applicants discovered the most efficient method for germline transmission and production of chimeric mice was "black in black combination.

MPEP 608.01(g) requires the applicants to describe the invention to enable any person skilled in the art or science to make and use the invention without involving experimentation. Applicants have submitted FOUR Examples and Eighteen Figures describing in detail the different embodiments of the invention. See Specification page line 7 to page 20, line 10. Specifically, Example 2 describes the Black in clack combination and Figures 8, 9 and 10 describe the results obtained.

Despite this, the Action states,(Page 8, lines 12-15) "*However, no guidance has been provided for why an artisan would generate a "black into black combination", "black into white", "white into black" mouse that is different from generating any transgenic mouse of any colored coat"*

Response: Applicants can only conclude from the above statement that this examiner has not reviewed applicant's application adequately. This may explain her piecemeal prosecution in the First Office Action, and factual mistakes discussed above.

The Action states (Page 8, line 15 to Page 9, line 4)-*While the Examiner has considered the Applicant's argument regarding coat color, as it applies to the Applicant*

overcoming the teachings of Scuster-Gossler (Applicant's Response, page 7, 5th paragraph), wherein unlike Schuster –Gossler, who uses white mice with a tyrosine mutation, wherein the tyrosine mutation has to be bred out of the mice, the Applicant does not provide evidence that teaches an artisan that the mice with the tyrosine mutation has deleterious effects on studying the phenotypes of a knockout mouse generated using blastocysts from C57BL/6J c2J mice. The applicant also points out that Schuster-Gossler states that the C57BL/6J c2J mice have low blastocyst production (Schuster-Gossler et al, page 1026, 1st cl. Last parag.). However, Schuster-Gosser then states that despite the low production of blastocyst, "adequate numbers can be obtained (Schuster –Gossler et al., page 1026, 1st col. Last parag.)".

Response:

In response, as described above, applicants have provided evidence for why the mutation for tyrosinase has deleterious effects and extra mating is needed to remove the tyrosinase mutation. Specifically, the tyrosinase (Tyr) gene encodes the enzyme tyrosinase that catalyses the conversion of L-tyrosine into DOPA (3,4-dihydroxyphenylalanine)-quinone. The albino mutation abrogates functional activity of tyrosinase resulting in deficiency of melanin pigment production in skin and retina. Tyr maps to a region in the central position of Chromosome 7 that contains a skin tumor-modifier locus. Saran et al. isolated the albino mutation in transgenic mice to assess a possible role of Tyr gene in two-stage skin carcinogenesis. Transgenic expression of the functional Tyr (Cys) allele in albino mice (Tyr (Ser)) caused a reduction in skin papilloma multiplicity, in four independent experiments and at three dose levels of DMBA (9,10-dimethyl-1, 2-benzanthracene). In vitro mechanistic studies demonstrated

that transfection of the Tyr (Cys) allele in a human squamous cell carcinoma cell line (NCI-H520) increases tyrosinase enzyme activity and confers resistance to hydrogen peroxide-induced oxidative DNA damage. These results provide direct evidence that the Tyr gene can act as a skin cancer-modifier gene, whose mechanism of action may involve modulation of oxidative DNA damage. (Saran A., et al. 2004, Oncogene 20:4130-4135).

Finally, the Action states (Page 9, lines 4-7) *" As it stands, the Examiner has not given any patentable weight to coat color. Unless the Applicant directs the Examiner to evidence as to why an artisan would generate a transgenic mouse comprising specific coat color, the C57BL/6 transgenic mouse generated by the Applicants are like any other C57BL/6 transgenic mouse".*

Response- Applicant do not understand the above rejection. Claims 3-8 are method claims for generating genetically modified C57 mice. Coat color is a genetic marker to test chimerism. In the present invention, where color changes were not helpful in detecting chimerism, PCR and Southern Blot techniques were used to evaluate the chimeras. See Specification page 15, lines 11-19; page 19, lines 2-12, Figures 9 and 10. The present invention is not aimed at generating specific coat color for transgenic mice, but, the invention provides a system for constructing an animal model for a disease involving a genetic defect by developing a non-human vertebrate animal ES cell line lacking the specific gene, developing a genetically modified mouse model whose genome comprises an introduced null mutation of the gene which said mouse exhibits, and using this model to screen therapeutics and other agents. See Specification page 5, lines 15-19.

In summary, there is no basis for sustaining the above rejection of claims 3-8, and therefore the rejection should be withdrawn.

Applicants request the Examiner and her Supervisory Patent Examiner, Dr. Ram Shukla, to grant an interview at their earliest convenience.

Respectfully Submitted,

Rashida A. Karmali

Rashida A. Karmali, Esq.
Reg. No. 43,705
Attorney for Applicants
99 Wall Street, 10th Floor
New York, NY 10005
Phone (212) 651-9653
Fax (212) 651-9654

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Rashida A. Karmali
Attorney for Applicants

Rashida A. Karmali
Signature

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